Microbial Signaling in Plant—Microbe Interactions and Its Role on Sustainability of Agroecosystems

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Chapter 1
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Abstract Sustainability in agroecosystems is governed primarily by the functional balance between soil processes and plant productivity. Microorganisms are key drivers of important soil processes such as nutrient recycling, and their activity directly influences the functional stability and sustainability of the soil ecosystem. In nature, microbes tend to function as functional guilds or communities, thereby creating a complex network of microbial interactions. Therefore, microbial signalling processes play an important role in communication within a particular functional guild or among different guilds. Numerous chemical compounds acting as signalling molecules in the soil-plant system have been identified. However, the understanding of how these molecules contribute to soil ecosystem stability and sustainability through inter- and intra-species chemical signalling is incomplete. In particular, it is known that chemical inputs in agroecosystems can suppress some microbes (e.g. nitrogen fixers), which can also reduce the interactions between microbes due to destruction of the signalling networks, consequently breaking the delicate balance of the soil ecosystem. Understanding the impact of microbial signalling processes on soil ecosystem sustainability is imperative if we are to address this issue. This chapter reviews the current knowledge on the mechanisms of microbial signalling in plant–microbe interactions and technical advances in identifying signalling pathways between plants and soil and also proposes avenue for future research in this field.

Keywords Microbial signalling • Ecosystem sustainability • Plant–microbe interaction • Chemical fertilizers

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1.1 Introduction

Soils are complex ecosystems, composed of both biotic and abiotic components with regular interactions between both these components to maintain ecosystem function. Bot and Benites (2005) defined a soil ecosystem as an interdependent life-support system, in which the abiotic components, air, water, minerals, and organic matter, function together and interact closely with their biotic components of macro and microorganisms. These biotic and abiotic components thus principally govern the sustainability of soil ecosystem by maintaining soil fertility, soil health, and plant productivity (van der Heijden et al. 1998; Tilak et al. 2005; Richardson and Simpson 2011; Ranjan et al. 2015).

Soil biotic components are key drivers of important soil processes such as decomposition of organic matter, nutrient recycling, detoxification of toxicants, and suppression of noxious and pathogenic organisms (Doran and Zeiss 2000; Singh 2015a, b). Such a wide variety of soil processes which are driven by biotic components define the composition and sustainability of the soil and environment (Bot and Benites 2005; Singh and Gupta 2016). The soil food web comprises of a community of organisms living all or part of their lives in soil with interdependency for sources of carbon and energy. The soil food web determines cycling processes of major elements across ecosystems and is also a better predictor of such processes than land use (de Vries et al. 2013).

The diversity and abundance of life that exists within the soil ecosystem is greater perhaps than in any other ecosystem. With 1g of soil holding up to ten billion microorganisms and thousands of different species (Knietsch et al. 2003), the impact of microbial, functional diversity cannot be underestimated. The extent of microbial diversity creates an intricate network of microbe–microbe and plant–microbe interactions with complex systems of intra- and inter-species communication (Lambers et al. 2009). Through their abundance, diversity, food-web trophic, and community interactions, soil organisms maintain the functional equilibrium of soil ecosystems (Doran and Zeiss 2000). As revealed by Lupatini et al. (2014), microbial species interactions in the soil food web could be more important to soil processes than just species richness and abundance. Such interactions have been reported to reduce inter-species competition and increase the number of coexisting species leading to an increase in biodiversity (Bastolla et al. 2009). In such biodiverse communities, an array of small hormone like molecules helps achieve both cell-to-cell signalling and communication between the microorganisms and their hosts (Hughes and Sperandio 2008). These chemical signaling compounds function as messengers for communicating among the diverse groups of organisms in the soil ecosystems for cooperative existence and are also attributable to the stability and balanced responses of a large number of individuals in the soil food web (Seneviratne 2015). Hence, understanding how these microorganisms maintain a correct balance between inter- and intra-species interactions is important for sustenance of soil ecosystems. There is at present an incomplete understanding of plant–microbial signaling compounds and the mechanisms underlying plant–microbe interaction in both symbiotic and

defence associations. In particular, the importance of chemical signaling in ecosystem sustainability is less documented and calls for further attention. Thus, this chapter highlights the fact that sustainability of soil ecosystem is an outcome of maintaining a robust signaling stability within soil microbes and also optimizing plant–microbe interactions.

1.2 Signalling Molecules in Plant–Microbe Interactions

Plant–microbe interaction is critical to maintain soil health and sustainability (Tak et al. 2013). Plant growth is influenced by microbial functional diversity through a variety of mechanisms, including biological nitrogen fixation by different classes of *Proteobacteria*, increased biotic and abiotic stress tolerance imparted by the presence of endophytic microbes, and direct and indirect advantages rendered by plant growth-promoting rhizobacteria (Barea et al. 2005). It has long been known that plants and microbes interact closely through the release of signaling compounds forming an array of symbiotic and defence associations.

To date, a plethora of such chemical compounds acting as signaling molecules in the soil-plant feedback system have been identified (Berg 2009; Ortíz-Castro et al. 2009; Mandal et al. 2010). Some of these signaling molecules are plant derived whilst some are from microorganisms and influence plant–microbe interactions, plant growth, and ecological balance (Lambrecht et al. 2000; Ortíz-Castro et al. 2009; Mabood et al. 2014; Ludwig-Müller 2015). The wide variety of signaling compounds produced by plants include both primary metabolites (carbohydrates, proteins, organic acids, etc.) and secondary metabolites (flavonoids, phenol, phytohormones, etc.) (Singh et al. 2016a, b). On the other hand, microorganisms in soil release small molecules or volatile compounds called phytohormones, which may act directly or indirectly to activate plant immunity or regulate plant growth and morphogenesis. The major classes of signals participating in the interactions that occur between plants and beneficial microorganisms are carried out by compounds which include phytohormones, *N*-acyl-L-homoserine lactones, volatile organic and secondary plant compounds, etc. (reviewed in Ortíz-Castro et al. 2009).

The signaling compounds play diverse roles in ensuring benefits to both parties of the plant–microbe interaction. Phytohormones, such as auxins and cytokinins, produced by either bacteria or fungi, can act as signaling molecules and affect cell proliferation or modify root system architecture by overproduction of lateral roots and root hairs with a subsequent increase of nutrient and water uptake (Ortíz-Castro et al. 2009). Of these two groups of compounds, the production of cytokinins by plant growth-promoting rhizobacteria has been well documented and correlated with an increased growth in plants (Nieto and Frankenberger 1990; Arkhipova et al. 2005; Ortíz-Castro et al. 2009). Cytokinins are also key regulators of intricate plant–microbe–insect interactions and contribute to plant growth-defence tasks of facing both mutualists and invaders (Giron et al. 2013). The synthesis of the other group of phytohormones namely auxins can take place via a diverse group of

bacteria during multiple pathways of their metabolism. These auxins include indole-3-acetic acid (IAA), the major naturally occurring auxin, indole-3-butyric acid (IBA), phenylacetic acid, 4-chlorindole-3-acetic acid, or their precursors (Spaepen et al. 2007). IAA in particular acts as a reciprocal signaling molecule in bacterial–plant interactions and some PGPR stimulate root proliferation by IAA biosynthesis (Lambrecht et al. 2000). As reviewed by Ludwig-Müller (2015), auxins also play a dual role in plant developmental processes; stimulation of cell division and cell elongation in healthy plants, and defence mechanisms by acting as defence molecules with antimicrobial activity. Many fungal species also produce auxins. Based on recent evidences, it has been suggested that fungi may use IAA and related compounds to interact with plants as part of its colonization strategy, which could primarily result in plant growth stimulation and modification of basal plant defence mechanisms (Ortíz-Castro et al. 2009). In a similar manner, bacterial volatiles such as acetoin and 2,3-butanediol produced by certain PGPR can be used as plant–bacteria communicators and as aforesaid plant growth promotion triggers (Ortíz-Castro et al. 2009). In addition to these compounds, some organic acids (e.g. citrate, oxalate, and malate) play a central role in aluminium tolerance mechanisms, such as the detoxification of aluminium in the plant rhizosphere by releasing organic acids that chelate aluminium (Ma et al. 2001).

Long-term close interactions between different biological species, such as symbiosis and pathogenesis, are common between plants and soil microorganisms. Among the astounding number of such mutualistic associations, the legume–rhizobia nitrogen-fixing symbiosis of plant–bacterial nature and that of mycorrhizae of plant–fungal nature are well documented. Such relationships rely largely on various signaling molecules to ensure their sustenance. For example, phenolic acids, the main polyphenols made by plants, carry out diverse tasks which include acting as signaling molecules in the initiation of legume–rhizobia symbioses, establishment of arbuscular mycorrhizal symbioses and acting as agents in plant defence mechanisms (Mandal et al. 2010). An assortment of secondary plant compounds such as flavonoids and strigolactones, the latter of which is excreted by roots, also carry importance as signaling molecules in several symbiotic and pathogenic plant–microbe interactions (Steinkellner et al. 2007). For example, plant roots release flavonoid compounds, which signal rhizobia to produce lipooligosaccharide, and details on rhizobia–legume communication and signal transduction pathways have been described by Garg and Chandel (2010). Also, in response to the secretion of signal molecules recognized to be plant hormones known as “strigolactones”, arbuscular mycorrhizal fungi penetrate and colonize plant roots (Haichar et al. 2014).

In nature, microbes tend to function as functional guilds or communities, sometimes comprising of billions densely packed cells. Biofilms, one such group of communities, are adherent cells embedded within a self-produced matrix of extra cellular polymeric substance (EPS). Coordination of metabolic interactions among such biofilms is known to occur predominantly through quorum sensing (Reading and Sperandio 2006). Quorum sensing is a form of cell-to-cell communication between bacteria mediated by small diffusible signaling molecules called autoinducers that increase concentration as a function of cell density; these generally vary

depending on the nature of bacteria as *N*-acyl-L-homoserine lactones (AHLs) for Gram-negative bacteria (Ortíz-Castro et al. 2009) and peptide-signaling molecules for Gram-positive bacteria (Walker et al. 2003). These bacteria also have a receptor that can specifically detect the autoinducer. When the microbial population grows the inducer reaches a threshold concentration activating the receptor which then causes specific genes to begin transcriptional activities at approximately the same time. These activities enable intercellular signals of a bacterial population to control the expression of genes in response to cell density. This coordinating behaviour of bacteria can be useful in a variety of situations. For example, quorum-sensing systems possessed by both Gram-negative and -positive bacteria, including important plant pathogenic bacteria such as *Erwinia* spp., *Pseudomonas* spp., and *Agrobacterium* spp., can control the expression of several genes required for pathogenicity as reviewed in Fray (2002).

AHLs play an important role in the quorum sensing of different species. For instance, it is used for regulating diverse behaviours in rhizosphere inhabiting bacteria where in some situations plants may produce their own metabolites which may interfere with quorum-sensing signaling (Ortíz-Castro et al. 2009). The responses to AHL also vary, where bacteria respond to AHLs via biofilm formation, production of virulence factors, and symbiosis with plants. On the other hand, the plant recognizes AHLs and responds by altering gene expression in roots and shoots, thereby modulating defence and cell metabolism, root architecture, hormone responses, protein processing, and cytoskeletal organization (Ortíz-Castro et al. 2009).

Apart from the customary cell-to-cell communication via quorum sensing, it has been reported recently that electrical signals like potassium ion-channelling can be used to coordinate metabolism and to communicate within the biofilm (Beagle and Lockless 2015; Masi et al. 2015; Prindle et al. 2015). Further, Turrà et al. (2015) reported that the catalytic activity of secreted class III peroxidases triggered directed growth of the soil-inhabiting plant pathogen *Fusarium oxysporum* towards the roots of the host plant tomato (*Solanum lycopersicum*). Thus, this wide array of signaling molecules and their specific functions within bacterial communities diversify the relationships in plant–microbe interactions, and their role within the soil food web can be further explored.

1.3 Microbial Coordination of Complex Network Interaction Within Soil Food Web and Plant–Microbe Interactions

It is a known fact that soil bacterial communities use species-specific quorum-sensing signals or auto-inducers to coordinate gene expression within them, according to the density of their local population. However, subsequent findings have identified non-species-specific auto-inducers that are capable of mediating intra- and inter-species communication among different bacteria (Galloway et al. 2012). The role of these autoinducers has been demonstrated by plant defence responses and root development (Bai et al. 2012). Some higher plants on the other hand were

shown to have interactions between them and soil bacteria by the production of bacterial auto-inducers or signal-mimic compounds (Teplitski et al. 2000). Berdy (2005) reported that endophytic microbes in higher plants are responsible for producing such mimic compounds and other metabolites. It is therefore evident that endophytes are bound tightly to biosynthetic pathways of secondary metabolites in the hosts. Plants can also detect molecules produced by potential pathogens and activate pathogen-response systems thus placing plant defence mechanism to be a common role of the secondary metabolites in plants. As such, we suggest that there could be a close communication between the plants and their endophytes for producing the secondary metabolites when the need arises, for example, in the case of a pest or pathogen attack. The flavonoid pathway in plants produces a diverse array of compounds with functions which include defence mechanisms against pathogens, signaling in symbiosis, auxin transport regulators, and roles as antioxidants and pigments (Hassan and Mathesius 2012). An example for such signaling functions is seen in alfalfa, where chemotaxis towards the host plant by symbiotic *Sinorhizobium meliloti* has been reported to mediate from the sensing of proline secreted by roots (Webb et al. 2014).

In the presence of host plant physiological stress, many eukaryotic signal molecules are released and detected by Gram-negative pathogenic bacteria which respond by adapting their physiology for virulence (Lesouhaitier et al. 2009). The two conflicting demands balanced by plant immune signaling networks in pathogenesis have been identified to be vigour against pathogenic perturbations and moderation of negative impacts of immune responses on plant fitness (Sato et al. 2010). Recent findings provide further evidence for the important role of microbial signaling, showing intriguing complex interactions mediated by signaling among plant–insect–microbe relationships. This can be seen in the aphid-mediated plant immunity against pathogen infection, where in particular the priming of defence responses against different pathogens through hormonal signaling has been found to help prepare the plant for subsequent pathogen attacks (Lee et al. 2012). Another exciting study reports that plants can exploit common mycorrhizal networks in the soil for herbivore-induced defence signal transfer and interplant defence communication to activate defence responses more rapidly and aggressively upon insect attack and to increase their insect resistance (Song et al. 2014). Thus, it is clear that it is the microbes living in association with plants and also in the soil that contribute to ecosystem balance through signaling in complex network interactions.

1.4 Regulation of Microbial Signalling Compounds by Biotic and Abiotic Factors

Organisms challenged by a change in the environment can respond to that by secreting common signaling compounds (Smith et al. 2015). The activation, alteration, diminution, or termination of some signaling components in organisms can be regulated by numerous biotic and abiotic factors. These factors include a complex

matrix of plant–microbe and microbe–microbe communications and various environmental changes, as shown in this section.

Altered temperature (Schwinghamer et al. 2015) and water stress (Prudent et al. 2015) are two main factors leading to release signalling compounds. The architecture of the root system, for example, undergoes modification by its endogenous auxin levels and by environmental stimuli such as the availability of water and mineral nutrients (López-Bucio et al. 2003; Pérez-Torres et al. 2008). Plant rootlets starved of soil nitrogen have been observed to secrete small peptides that are translocated to the shoot and received by specific receptors so that the signaling from the root to the shoot helps the plant adapt to fluctuations in local nutrient availability (Tabata et al. 2014). In this instance, the signaling may induce the action of endophytic nitrogen fixers for compensating the deficiency in supply of soil nitrogen to the plant (Seneviratne 2015).

Moreover, in response to high doses of UV-B radiation, an induction of signaling molecules such as abscisic acid (ABA), nitric oxide (NO), and calcium ions (Ca^{2+}) in plant and animal cells can occur to bring about stress tolerance (Tossi et al. 2012). It has also been reported by Flores et al. (1999) that an insecticidal defence response can be created by UV light penetrating soil layers leading to photo-activation of fluorescent β-carboline alkaloids secreted by oca roots. Another example of such a response has been reported by Ma et al. (2001) in maize and wheat, where the exudation occurs of some known signaling molecules such as citrate, malate, and related organic acids in response to high Al^{3+} concentrations. Further, significant physiological functions are implemented by NO that modulates the activities of cellular and extracellular proteins in various groups of organisms (Medinets et al. 2015). Nitric oxide can further play a signaling function to enhance microbial biofilm formation in the soil (Medinets et al. 2015), which renders numerous biochemical and physiological benefits to plant growth (Qurashi and Sabri 2012).

Beneficial soil bacteria confer immunity against a wide range of foliar diseases by activating plant defences, thereby reducing a plant's susceptibility to pathogen attack. This is clearly seen in the reporting of root secretions of L-malic acid which is induced by the foliar pathogen *Pseudomonas syringae* pv *tomato* and in the elevated levels of L-malic acid, which promote binding and biofilm formation of beneficial rhizobacterium *Bacillus subtilis* on Arabidopsis roots (Rudrappa et al. 2008). In addition, it has been observed that biofilm formation improves soil fertility through aggregate formation (Qurashi and Sabri 2012) and carbon storage (Seneviratne et al. 2011), which in turn govern the sustainability of the soil ecosystem.

1.5 Signalling Pathways in Soil Food Web Improve Ecosystem Functioning and Sustainability

Microbes play an important role in chemical signaling in plant–microbe interaction as discussed above. Thus, microbial signaling pathways in soil and plant–microbe interactions improve ecosystem functioning and sustainability (Fig. 1.1).

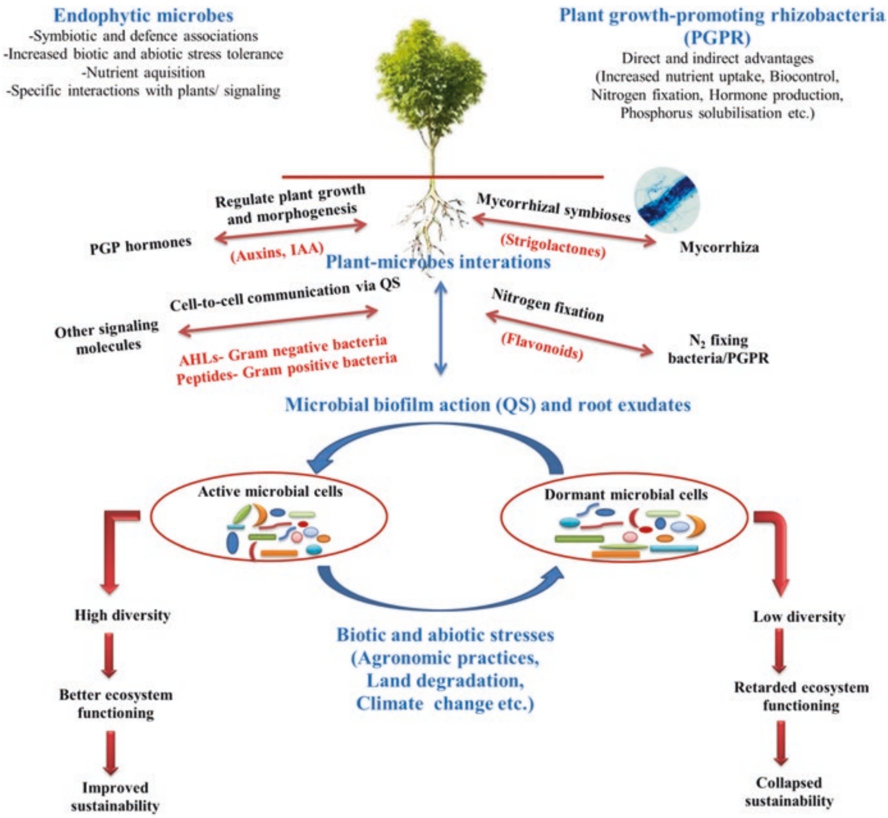

Fig. 1.1 Microbial signaling pathways in soil and plant–microbe interactions improve ecosystem functioning and sustainability in the long term

Beneficial microbes in plant and rhizosphere, such as endophytes and plant growth-promoting rhizobacteria (PGPR) stimulate plant growth though a wide range of mechanisms. Some direct and indirect advantages of PGPR and endophytes include: (1) increased nutrient uptake, (2) biocontrol, (3) nitrogen fixation, (4) hormone production, (5) phosphorus solubilization, (6) facilitation of symbiotic and defence associations, (7) increased biotic and abiotic stress tolerance, etc. (Smith et al. 2015).

On the other hand, root exudates of plant origin, which act as signaling molecules in plant–microbe interactions, are one of the key drivers of soil microbial community composition, diversity, and functional richness within the rhizosphere. Plant–microbe interactions in the rhizosphere create a highly structured and active microbial community and this ultimately leads to better ecosystem functioning and improved sustainability.

The knowledge that microbial signaling play as an integral part in plants and animals has also been confirmed (Seneviratne 2015). Microbes in macro-organisms provide their metabolic activity producing an amazing diversity of compounds and

signaling molecules for nutrition, protection, and development of the hosts (Selosse et al. 2014). However, the functional role of microbes-mediated chemical signaling in soil ecosystem sustainability is less documented. Also, the understanding of how these molecules contribute to soil ecosystem stability and sustainability through inter- and intra-species chemical signaling is limited. Seneviratne (2015) established an idea of the linkage between ecosystem sustainability and microbial chemical signaling, thereby introducing a new term edaphic ecosystem signal transduction (EST). The EST is defined as chemical signaling from signaling molecules to trigger a change in the activity or state at and within the edaphic ecosystem, considering the ecosystem to be a single unit like a cell. According to this concept, the EST is mediated by receptors of microbes in soil, plants, and animals of the ecosystem through a cascade in signal-receptor-process-response, thus leading to maintenance of a delicate balance among the interacting counterparts in the edaphic ecosystems. The resultant response could be a signal which again triggers receptors of other counterparts of the ecosystem, thereby instituting a signaling network.

However, when the signaling network is disrupted due to human impact, the sustenance of soil ecosystems collapses {e.g. chemical inputs in agricultural practices (Fox et al. 2007), tillage, and global change}. This can be seen in the example of nitrogen fixers who play a key role in the growth and persistence of effective microbial communities in the soil by supplying nitrogen through biological nitrogen fixation (Seneviratne et al. 2011). Here, a disruption of signaling networks occurs upon the use of nitrogenous fertilizers in agriculture and forestry which lead to a suppression of the action of microbes, particularly nitrogen fixers in agroecosystems. This tends to produce nitrogen-deficient, weak microbial communities with low biomass and activity, due to diminished nitrogen supply from the repressed nitrogen fixers, which paves the way to collapsed microbial diversity and ecosystem functioning. Under this circumstance, two negative impacts can be observed in the ecosystem, namely, (1) reduced soil fertility and organic matter build up (Scholes and Scholes 2013; Bi et al. 2015) leading to low soil moisture retention, and hence drought stress, and (2) yield decline (Kumar and Yadav 2001), possibly due to lack of rhizoremediation, resulting in phytotoxin accumulation (Dams et al. 2007), which are prevalent in collapsed sustainability. These effects can be minimized or restored by manipulating soil microbial diversity in the ecosystems for re-establishing communication through EST, but not solely from nutrients and water management, as suggested in conventional agriculture and forestry (Seneviratne 2015).

1.6 Technical Advances in Identifying Signalling Pathways in Soil-Plant System

The rhizosphere, the soil in the immediate vicinity of growing plant roots, is a complex, structured, and dynamic system with myriads of microorganisms. The relative abundance of particular microbial species that interact with plants can be shifted within different plant species and their genotypes, and also in response to both

abiotic and environmental factors. Therefore, assessment of relative abundance, taxonomic diversity, and functions of plant associated microorganisms are vital to reveal unknown signaling pathways between plant and soil microbes. This can be achieved successfully through an integrated approach of currently available molecular tools. For example, recent advances in multi-omics techniques, particularly in analytical capabilities (NMR, GC-MS), that aid detection of metabolites in environmental sample at low concentrations can be employed to improve our knowledge on microbial signaling. Whilst a detailed review of the techniques is out of scope for this chapter, we provide an overview of the techniques and its application to study plant–microbe interactions.

Majority of the microorganisms in the environment are recalcitrant to isolate and grow in the laboratory and thus cultivation-dependent techniques offer a very limited view of the microbial diversity and function. The advent of cultivation-independent techniques, i.e. nucleic acid-based evaluation of microbial diversity initiated a paradigm shift in our understanding of the microbial world. These cultivation-independent techniques rely on the extraction of nucleic acids directly from the environmental samples, such as soils, followed by either amplicon or shotgun sequencing to profile the microbial community. Traditionally, amplicon sequencing was performed on PCR-amplified marker genes using specific set of primers targeting either the 16S rRNA gene or metabolic genes, to infer phylogeny. Recent advances in sequencing chemistries has enabled researchers to use high-throughput sequencing (HTS), which provides an advantage over traditional Sanger sequencing and have resulted in a better understanding of microbial functional diversity in the environment, particularly plant–microbe interactions (Knief 2014). Amplicon sequencing is a cost-effective HTS technique to assess the taxonomic diversity of microbial community in an environment (Di Bella et al. 2013). HTS of marker genes has been used for characterization of the microbial community composition in phyllosphere and rhizosphere (Jiang et al. 2013; Bokulich et al. 2014; Bulgarelli et al. 2015) and to address key questions such as whether or not plant taxa select their microbial community composition (Delmotte et al. 2009; Bokulich et al. 2014) and how microbial community composition differs among different plant compartments (Bodenhausen et al. 2013; Ottesen et al. 2013).

Shotgun sequencing of the community DNA, i.e. metagenome, allows the researchers to not only identify the taxonomic diversity, but also to access the metabolic blueprint of the microbial community, i.e. genes and their functionalities (Di Bella et al. 2013). Moreover, shotgun metagenomes eliminate primer bias, i.e. sequencing without the need for targeting and amplifying a particular marker gene (Poretsky et al. 2014). Researchers have employed shotgun approaches to characterize phyllosphere microbial communities in rice, clover, soybean, and tomato (Delmotte et al. 2009; Atamna-Ismaeel et al. 2012; Knief et al. 2012; Ottesen et al. 2013). Using shotgun sequencing, Mendes et al. (2014) reported that rhizosphere microbiome in Soybean is a subset of the microorganisms observed in the bulk soil.

Global metagenome datasets are available in public repositories (JGI, MG-RAST, NCBI) and can be used to mine for genes that are identified to be involved in microbial signaling pathways. Whilst metagenomes can provide us information on the

genes which do not necessarily indicate activity. Transcriptomics allows the analysis of messenger RNA (mRNA) molecules, or gene transcripts, produced by an isolate or a specific gene (targeted transcriptomics) or a microbial population in an environment (meta-transcriptomics) at a specific developmental stage or physiological condition (Wang et al. 2009; Zhang et al. 2010). The mRNA represents the template for protein synthesis and the genes that respond the environmental stimuli are actively expressed and are reflected in the transcriptome (Horgan and Kenny 2011). Advances in the RNA sequencing techniques (RNA-seq) have significantly transformed the analysis of microbial transcriptomes (Croucher and Thomson 2010). RNA-seq can be used to determine gene expression levels and their dynamics across different microbial cells or induced environmental stimuli. Understanding microbial signaling pathways, in isolates or in co-culture experiments, will be aided by this technique by comparing up- or down-regulated genes in response to a specific treatment or stimuli and identification of specific groups of molecular processes, in this case genes that are involved in signaling pathways (Wit et al. 2012).

Proteins can also be used as biomarkers for biological functions as they represent the activity of metabolic reactions and provide more information on specific microbial processes (Keller and Hettich 2009). With recent advances in protein extraction techniques from environmental samples, proteomic techniques, and databases, it is now feasible to identify at least 50–70 % of a predicted bacterial proteome (Keller and Hettich 2009; Branca et al. 2014; Meyer et al. 2014; Yang et al. 2015). Whilst a strong correlation between mRNA expression levels and protein abundance can be assumed (Zhang et al. 2010), several studies have reported that it is not the case (Taniguchi et al. 2010; Vogel and Marcotte 2012). Therefore, it is essential to use transcriptomics and proteomics in tandem to obtain insights into microbial functions (Zhang et al. 2010). Whilst the metaproteome represents the composite of all proteins recovered from an environmental sample, metaproteomics has been applied to profile microbial community and function in various environmental samples, for example, soils (Bastida et al. 2014; Wang et al. 2010), freshwater (Habicht et al. 2011; Hanson et al. 2014), marine environment (Sowell et al. 2011; Stokke et al. 2012), and plants (Delmotte et al. 2009; Knief et al. 2012).

Further, metaproteomics can be used to study plant–microbe interactions, yet certain technical challenges remain in the separation of plant and microbial materials (Delmotte et al. 2009; Knief et al. 2012), as the microorganisms associated with the plants may not be well characterized (both isolates and the availability of isolate genomes). Therefore, protein identification depends on either the availability of whole genomes from related microbes or a metagenome from the same sample. Using metagenome in tandem to metaproteome significantly enhances the identification of peptides and its phylogenetic affinity (Delmotte et al. 2009; Knief et al. 2012). Thus, in the future, metaproteogenomic (combining metaproteomics and metagenomics) will be an integral part of researchers' tool kit intending to unravel the impact of microbial signaling on ecosystem health and productivity.

Environmental metabolomics is the application of metabolomics to analyse endogenous and exogenous low molecular mass metabolites in the environment to study organismal interactions as reviewed in Lankadurai et al. (2013).

Metabolomics is an excellent platform to detect metabolites, i.e. signaling molecules in the environment and understand the impact of environmental stimuli on microbial signaling. Researchers have used metabolomics to understand the response of microbes to external stress such as heavy metal or organic pollutants (Marles-Wright and Lewis 2007; Ye et al. 2012).

1.7 Conclusions and Future Perspectives

A better understanding of microbial signaling pathways is a key to success in manipulating beneficial plant–microbe association for better nutrient management (e.g. biofertilizers), suppression of pathogens (biopesticides), and crop stress alleviation which paves the way to environmental sustainability. Soil microbes maintain a complex interaction with other micro- and macro-organisms in the soil food web and plants via various signaling mechanisms. These communications are vital for nutrient assimilation, development, and activation of defence mechanisms in positive microbe–plant interactions. In addition, plant stress responses play an important role in the release of signaling compounds in the rhizosphere, and a better understanding of the relationship between environmental plant stresses and signaling could help in developing technologies that utilize plant signaling in crop stress alleviation (Barea 2015; Smith et al. 2015). Further, variable environmental factors may account for some of inconsistencies observed in field trials, and hence a more complete understanding of how plant–microbe communication is influenced by environmental factors would be beneficial (Smith et al. 2015). Recent advancements in molecular biology including the development of next generation sequencing approaches (e.g. such as metatranscriptomics, metagenomics, proteomics, metabolomics) means it is now possible to understand these microbial signaling mechanisms based on common genes, signalling pathways, and systems in a variety of ecosystems. Therefore, there is an increasing interest in the use of “multi-omics” approaches leading to improved mechanistic models of microbial community structure and function across soil ecosystem and plant–microbe interactions. This helps to upgrade our current knowledge in microbial signaling pathways and the factors that regulate the signalling in the soil ecosystem. This understanding can be used to manipulate the beneficial associations in disturbed ecosystems like croplands, particularly in agroecosystems where chemical inputs weaken the interactions through collapsing the signaling networks, consequently breaking the delicate balance of the ecosystem. Thus, bridging the knowledge gaps in microbial signaling in soil and plant–microbe interactions can no doubt lead to sustainable agricultural practices by developing more effective, low-cost, and eco-friendly agricultural practices.

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